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(54) Title: BIOENGINEERED HUMAN BLOOD CELLS			
(57) Abstract			
<p><i>In vitro</i> production of clinically useful quantities of mature, differentiated human blood cells by a method in which human pluripotent hematopoietic stem cells, preferably from a universal donor, are incubated in a bioreactor in a growth medium also containing specific recombinant human growth and maturation promoting polypeptide factors in combinations that expand stem cell cultures and promote the maturation and differentiation of stem cells into erythroid, thrombocytic or granulocytic human blood cells, and harvesting the mature cells. The growth and maturation promoting polypeptides employed include SCGF, Interleukins 1,3,4,5,6, and 11, GM-CSF, M-CSF, G-CSF and EPO. Stem cells may be modified so as to remove histocompatibility and/or blood group antigens, or may be genetically altered by transfection with appropriate DNA-containing vectors, prior to addition to the bioreactor. Erythrocytes prepared in large quantities by this method are a good source of iron, as iron-saturated hemoglobin, for use in iron replacement therapy.</p>			

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BIOENGINEERED HUMAN BLOOD CELLS

Background of the InventionField of the invention

5 This invention relates generally to the *in vitro* production of human blood cells, and more particularly to the use of recombinant human growth and maturation promoting polypeptides to produce clinically useful quantities of mature blood cells from human pluripotent hematopoietic stem cells.

10 Description of the background art

Despite advances in blood typing and in testing for the presence of infectious agents in blood, the use of donated human blood for transfusions remains fraught with danger. Even with typing and cross-matching, there continues to be major risks with blood transfusions including febrile or urticarial reactions (1:100), non-fatal hemolytic reactions (1:25,000) and fatal hemolytic reactions (1:1x10⁶) (Epstein, Increasing the Safety of Blood Transfusions, American Red Cross, 1992, p.1)

20 The other major risk of modern blood transfusion is the transmission of infectious agents. Currently, the risk of contracting HIV infection from heterologous blood transfusions has been estimated at between 1:40,000 and 1:150,000 (Epstein, 1992, above). The other major, and occasionally fatal, blood-borne infection is hepatitis, with the risk of contracting hepatitis B infections estimated at less than 1:250,000, and the risk of hepatitis C (non-A, non-B) calculated at 1:500 - 1:3,000 (Epstein 1992, above). Other less common but periodically significant infectious agents include HIV-2, HTLV-1 and HTLV-2 (less than 1:1x10⁵), CMV (variable), Yersinia enterocolitica (less than 1:1x10⁶), and rarely Trypanosoma cruz; (Chagas' disease), human parvovirus B19, Borrelia burgdorferi (Lyme disease), Treponema pallidum (syphilis), plasmodium virus and falciparum (malaria), and human herpes virus type 6 (HHV-6), (Epstein, 1992, above).

35 Because of these risks, there is an important need for safe alternatives for blood transfusions. Native

hemoglobin has been chemically modified by various methods in an attempt to create a blood substitute, but thus far such products suffer from a variety of shortcomings, including nephrotoxicity, excessive O₂ affinity due to loss of 2,3-diphosphoglycerol, a short half-life (usually 4-6 hours), rapid dimerization and excretion, and insufficient plasma concentration (Skolnick J. Amer. Med. Assoc. 268:697 (1992); Vigerou et al., Bull. Acad. Natl. Med., 174:947 (1990).

Human hemoglobin has been packaged in liposomes for administration as neo-erythrocytes, but such products are difficult to sterilize (particularly against viruses such as HIV), they exhibit a short half-life because they are rapidly cleared by the reticuloendothelial system, and significantly suppress the immune system, thereby predisposing recipients to an increased infection rate (Djordjeric et al, Crit. Rev. Ther. Carrier Syst., 6:131 (1989).

Perfluorochemicals, (e.g., Fluosol-DA) have been tested as hemoglobin substitutes, but these perfluorocarbons contain a potentially toxic surfactant (Pluronic F-68), they must be stored frozen, and, due to their insolubility, require emulsification. In addition, these fluids require oxygen-enriched (potentially toxic) air for proper oxygen delivery, as well as frequent administration due to a short half-life (Skolnick, 1992 above; Vigeron et al, 1990, above).

It is clear that, despite these efforts, an effective and safe blood substitute is still not available. The Applicant has determined that an attractive alternate approach is, not to develop a substitute blood, but rather to produce clinically useful amounts of natural, mature, differentiated, universally compatible human blood cells under conditions such that the major risks from blood-borne infectious agents and transfusion reactions are absent to insubstantial. Such an approach has been invented, and is described below.

Summary of the Invention

The invention consists of a method for *in vitro* culturing of pluripotent hematopoietic stem cells taken from a human donor so as to produce clinically useful amounts of infectious agent-free, universally compatible human blood cells. The stem cells are cultured in the presence of recombinant human growth and maturation promoting factors, such as cytokines, lymphokines, colony stimulating factors, mitogens, growth factors, and maturation factors) so as to produce at will clinically useful quantities of particular infectious agent-free human blood cells such as erythrocytes, megakaryocytes, monocytes, macrophages, neutrophils, eosinophils, basophils, platelets, as well as expanded stem cell cultures. The human donors are preferably universal donors, i.e., blood type O, Rh factor negative.

It is an object of this invention to provide a bioreactor system, such as those containing a ceramic matrix core, hollow capillary fibers or protein coated microspheres, in order to expand the numbers of human pluripotent stem cells, by inoculating such bioreactors with human bone marrow, either in its entirety or variably purified, and subsequently perfusing the culture chamber with growth medium containing human recombinant growth and maturation promoting polypeptide factors.

It is another object of this invention to provide a method for producing in clinically useful quantities universal donor (O-) erythrocytes from O- donor stem cells expanded in a bioreactor in the presence of human recombinant growth and maturation promoting polypeptides for transfusion into patients, e.g., into anemic or thrombocytopenic patients.

It is yet another object of the invention to provide a method for the expansion of human bone marrow cells modified by antigenic modulation techniques so as to eliminate cell surface antigens, e.g., human histocompatibility antigens and blood group antigens, such that more universal bone marrow precursor cell cultures

can then be expanded for the production of universal bone marrow cells for bone marrow transplantation, as well as for the production of erythrocytes, platelets, leukocytes and other mature blood cells for transfusion purposes.

5 Yet another object of this invention is the provision of a method of gene therapy for the treatment of congenital or acquired genetic diseases by the expansion of genetically altered bone marrow precursor cells in bioreactors until cell numbers are large enough for
10 reinfusion into the donor patient.

It is another object of this invention to provide a method for producing large amounts of iron-heme complexes that may be collected, purified, and used as an improved source of iron for clinical administration.

15 These and other objects will become apparent by reference to the specification and appended claims.

Description of the Figures

Figure 1 shows an overview of hematopoietic development as influenced by human growth and maturation
20 promoting polypeptide factors.

Detailed Description of the Preferred Embodiments

The Applicant has invented a method for expanding human hematopoietic stem cell cultures and for producing from such cells clinically useful quantities of infectious
25 agent-free, universally compatible human blood cells by culturing such bone marrow cells in a bioreactor together with particular human recombinant growth and maturation promoting polypeptide factors, and harvesting the thus-produced human blood cells. The expression "blood cells"
30 is intended to include erythrocytes (red blood cells), reticulocytes, megakaryocytes, eosinophils, neutrophils, basophils, platelets, monocytes, macrophages and granulocytes. For purpose of transfusion into patients, erythrocytes, granulocytes and platelets are particularly
35 valuable. By the expression "clinically useful quantities (or amounts) of blood cells" is intended to mean quantities of blood cells of whatever type sufficient for

transfusion into human patients to treat a clinical condition.

By obtaining hematopoietic stem cells from a universal donor (i.e., blood type O, (Rh factor) negative), the cells produced according to the invention may be transfused into nearly all patients.

For the purposes of this specification and the claims, the following abbreviations and definitions will be used:

- 1) Hematopoiesis: The process of forming and developing the various types of circulating blood cells and blood formed elements from immature stem cells.
- 2) Pluripotent stem cells: primordial cells that may differentiate into various specialized types of cells.
- 3) Stem cell (hemocytoblast): A primitive blood cell having the ability to differentiate into cells of the granulocytes series, (e.g., promyelocytes, neutrophils, eosinophils, and basophils), the erythrocyte series (reticulocytes, erythrocytes), the thrombocyte series (megakaryoblasts, platelet-producing megakaryocytes, platelets), and the monocyte series (monocytes and macrophages).
- 4) Landsteiner blood groups: A, B, A/B and O (null)
- 5) O-: Blood devoid of types A, B or A/B, and Rh factor negative. Cells from such blood are considered universal donor blood cells as they are compatible with nearly all major blood types in recipient patients.
- 6) Bone marrow: A hematopoietic organ that generates billions of blood cells daily.
- 7) Growth and Maturation Promoting Polypeptides ("GMPP"): Generic name for polypeptide cell growth, differentiation, and maturation promoting substances including, but not limited to, cytokines, lymphokines such as the interleukins, colony stimulating factors, mitogens, growth factors such as platelet growth factor, and differentiation factors.
- 8) PPSC: Pluripotent stem cell.
- 9) CFU-GEMM: Colony forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte.

- 10) CFU-Meg: Colony forming unit-megakaryocyte.
- 11) CFU-GM: Colony forming unit-granulocyte-macrophage.
- 12) CFU-Eo: Colony forming unit-eosinophil.
- 5 13) CFU-E: Colony forming unit-erythroid.
- 14) CFU-Bas: Colony forming unit-basophil.
- 15) GM-CSF: Granulocyte-macrophage-colony stimulating factor.
- 16) G-CSF: Granulocyte-colony stimulatory factor.
- 10 17) M-CSF: Macrophage-colony stimulating factor.
- 18) EPO: Erythropoietin, erythrocyte maturation factor.
- 19) SCGF: Stem cell growth factor (also known as mast cell factor and steel factor).
- 15 20) IL-1 TO IL-11: Interleukins (lymphokines).

Most preferably, the bone marrow of adult humans of blood type O and Rh factor negative is the source of the PPSC used in the present invention.

The invention is based on the fact that the processes of hemotopoiesis from PPSC, (i.e., self-renewal), proliferation and differentiation, are controlled by specific growth and maturation promoting polypeptide substances as defined above and as abbreviated hereinafter as "GMPP". The various GMPPs act at different steps along the path to committed mature blood cells (see Figure 1). PPSC, under the influence of SCGF, IL-1, IL-3 and IL-6, is converted to a myeloid stem cell, also referred to as CFU-blast cells. Under the additional influence of cytokines GM-CSF and IL-3, CFU-blast cells differentiate into progenitor CFU-GEMM cells. CFU-GEMM cells, under the influence of GM-CSF and IL-3 differentiate into five groups of progenitor cells namely, BFU-E (erythroid series), CFU-Meg (thrombocytic series) and CFU-GM, CFU-Eo and CFU-Bas (granulocyte series).

BFU-E, under the influence of IL-3, GM-CSF and EPO, differentiate first into CFU-E and then into mature red blood cells. CFU-Meg, under the influence of IL-3, GM-CSF and IL-11, differentiate first into platelet-forming

megakaryocytes and then into mature platelets. Platelets, themselves, secrete polypeptide growth factors referred to as PDGF (platelet-derived growth factor). CFU-GM is converted to either monocytes or granulocytes under the influence of M-CSF and GM-CSF (monocytes), and G-CSF and GM-CSF (granulocytes), respectively. IL-5 and GM-CSF convert CFU-E cells into mature eosinophils. Finally, IL-3 and IL-4 promote the differentiation of CFU-Bas cells into mature basophils.

According to the invention, production of clinically useful quantities of red blood cells, platelets, monocyte/macrophages, neutrophils, eosinophils and basophils, as desired, is accomplished by incubating PPSC cells in a bioreactor in a controlled environment with a growth medium containing combinations of particular recombinant human GMPPs. For example, for the production of mature red blood cells, PPSC cells are contacted with the combination of SCGF, IL-1, IL-3, IL-6, and GM-CSF, and with EPO as the terminal differentiation factor. For the production of platelets, PPSC cells are incubated with SCGF, IL-1, IL-3, IL-6, GM-CSF and IL-11.

Typical protocols are described below.

BONE MARROW PREPARATION

As a preferred method, heparinized bone marrow is obtained by aspiration from iliac crests of a human, most preferably O-, donor by conventional surgical techniques. At the same time, one unit (500 ml) of the donor's peripheral blood is collected in ACD blood bags, and the blood stored cold for later use. Reticulum is removed from the bone marrow by filtering the marrow through a 100 micron nylon mesh. This filtered bone marrow may be used immediately without further processing. It may also be processed by conventional techniques, (e.g., Ficoll-Hypaque centrifugation, counter-current cell elutriation, monoclonal antibody-magnetic bead separations) in order to isolate general populations such as low density cells or more-purified cell populations such as CD34+, CD71-cells (see, e.g., Sutherland et al., Blood 74: 1563

(1989); Smeland et al. Leukemia 6:845 (1992). Or, it may be preserved by incrementally freezing the bone marrow using computerized cryotechnology equipment (see U.S. Patent Nos. 4,107,937 and 4,117,881), and storing the cells at -196°C (liquid N₂).

CULTURE MEDIUM

A preferred growth medium for long-term culture of bone marrow cells consists of the AIM-V medium (Gibco, Grand Island, NY) supplemented with recombinant insulin (HUMULINTM, Eli Lilly & Co., Indianapolis, IN) at 10 µg/mL, human albumin (American Red Cross, Washington, D.C.) 50mg/mL, saturated human ferritin (T5391, Sigma Chem. Co., St. Louis, MO) at 200 µg/mL, hydrocortisone (sodium succinate derivative, The Upjohn Co., Kalamazoo, MI) at 10⁻⁶M, cholesterol (C3045, Sigma Chemical Co.) at 7.5 µg/mL, and Liposyn II (10%, Abbott Labs., No. Chicago, IL) at 0.05 mL/mL medium. In addition, penicillin G potassium (Roerig div. of Pfizer, Inc., New York, NY), gentamicin sulfate (Schering Corp., Kenilworth, NJ) and amphotericin B (Apothecon subsidiary of Bristol-Myers-Squibb, Princeton, NJ) are added to the cells as preservatives. Although AIM-V is preferred, other appropriate culture media may be used, such as Iscove's modified Dulbecco, Fisher's or Eagle's media. In addition, fetal calf serum or horse serum may be substituted for human serum albumin.

Recombinant SCGF (Systemix, Palo Alto, CA) at 10-1000 U/mL, recombinant human IL-1 (Amgen, Thousand Oaks, CA) at 1-100 U/mL, recombinant human IL-6 (Amgen) at 10-1000 U/mL, recombinant human IL-3 (Amgen) at 10-10,000 U/mL, and recombinant human EPO (Amgen) at 10-10,000 U/mL are added to the growth medium. In addition, recombinant human GM-CSF (Immunex, Seattle, WA) 1-100 U/mL is present in all cultures that are producing cells of the granulocyte series, erythrocytes series, and thrombocyte series. In addition, cultures are supplemented with recombinant human IL-5 (Arai, Ann. Rev. Biochem 59:1783 (1990) at about 10-10,000 units/mL when eosinophils are to be produced, recombinant human IL-4 (Arai, above) at

10-10,000 units/mL when basophils are to be generated, and recombinant IL-11 (Genetics Institute, Boston, MA) at 10-10,000 units/mL when megakaryocytes and platelets are to be produced.

5 BIOREACTORS

OPTICELL™ OPTICORE™ ceramic core S-51, S451 (flat surface area 23.8m²), S-1251 (flat surface area 10.4m²) or S-7251 (Cellex Biosciences, Inc., Minneapolis, MN) are preferred. These bioreactors are initially sterilely perfused, preferably for 1-3 days, with sterile deionized water to remove any toxic substances adhering to the core. Thereafter, the core is perfused for a brief period (less than 24 hours) with sterile 25% (w/v) human serum albumin in order to coat the core with protein. The bioreactor core is next perfused for 4-24 hours with a sterile solution of an anticoagulant, preferably heparin sulfate, 100 U/mL (Upjohn Co.) as a source of glycosaminoglycan and to prevent cell clumping during bone marrow inoculation. Following this preparation, the core is conditioned by perfusing it with sterile human bone marrow medium (see CULTURE MEDIUM above), preferably for about 24 hours, prior to inoculating the bioreactor with bone marrow.

BIOREACTOR CULTURE SYSTEM

The culture system consists of a variable number of bioreactors connected to the medium source by sterile plastic tubing. The medium is circulated through the bioreactor with the aid of a roller or centrifugal pump (e.g., KOBETM). Probes to measure pH, temperature, and O₂ tension are located in line at points immediately before and following the bioreactor(s). Information from these sensors is monitored electronically. In addition, provision is made for obtaining serial samples of the growth medium in order to monitor glucose, electrolytes, GMPP factors and nutrient concentrations. Activities of GMPPs are measured by conventional bioassays (e.g., bone marrow colony assays or dependent cell line growth assays) or conventional immunoassays (see, e.g., R & D Systems, Inc., Minneapolis, MN 55413).

INOCULATION WITH BONE MARROW CELLS

A number of bone marrow cells appropriate to the size of the bioreactor, at a concentration of about 2×10^7 cells/mL, are mixed with an equal volume of autologous fresh whole blood and injected into the bioreactor. Circulation of the growth medium is interrupted for a period of about 1-4 hours in order to permit the cells to attach to the surface of the bioreactor core or capillaries. Thereafter, the circulator pump is engaged and the growth medium pumped through the system at an initial rate determined by the size of the reactor; a typical rate is about 24 mL/min. Gas exchange occurs via silicone tubes (surface area = 0.5 m^2) within a stainless steel shell, or by a conventional membrane oxygenator. O_2 tension and pH are monitored continuously by polarographic O_2 probes and autoclavable pH electrodes, respectively. Flow rates are adjusted so as to maintain an optimal O_2 tension (a partial pressure of at least about 30-50mm of Hg) and optimal pH (7.30-7.45).

When an appropriate number of cells has been obtained, as determined by oxygen utilization of the system, a second bioreactor may be connected to the system, and cells fed directly into this second bioreactor. Thereafter, the second bioreactor is flushed with growth medium containing a high concentration (e.g. 10,000 U/mL) of EPO or other differentiation factor, and maintained for 1-3 days for final maturation of the desired blood components.

CELL HARVESTING AND PROCESSING

The bioreactor(s) is (are) mated with a conventional cell separator, and the cells are collected from the core or capillaries with gentle agitation. Harvested blood cells are processed in an automated cell separator and placed in sterile blood bags (American Red Cross) for later transfusion.

Bags of mature red blood cells are irradiated conventionally in order to inactivate any contaminating lymphocytes or other nucleated cells, and to improve

sterility. Bags are refrigerated for a period of 3-5 days prior to release. During this period, the cells may be tested for undesirable contaminants, such as infectious particles.

5 ADDITIONAL EMBODIMENTS

 It is now understood that iron-saturated hemoglobin (Fe-Hb) is a good source of iron for intravenous administration to patients. The red blood cells produced according to the invention may be lysed with a hypotonic solution, and the released Fe-Hb collected sterilely and
10 formed into complexes for intravenous use.

 The system described above may also be used to expand the numbers of human pluripotent bone marrow stems cells by inoculating a bioreactor with human mononuclear bone
15 marrow cells, either native or variably purified by conventional techniques, continuously perfusing the culture chamber with complete growth medium containing SCGF, IL-3 and IL-6, and harvesting the stem cells. By this means, bone marrow cells sufficient for human
20 transplantation purposes may be achieved.

 Culture-expanded bone marrow cells may be modified by pretreating donor cells in order to remove cell surface antigens, such as histocompatibility antigens or blood group antigens. By this means, more-universal bone marrow
25 progenitor cells for transfusion or for expansion as described above into mature blood cells may be produced. This may be accomplished, for example, by gene deletion mutations. Techniques to generate recessive loss-of-function mutations in genes by homologous recombination
30 and targeted integration are known in this art (see, e.g., Rossant et al, Phil. Trans. R. Soc. Lond. B 339:207 (1993); Capecchi et al, Science, 244: 1288 (1989); Hutchinson et al, Mut. Res. 299:211 (1993). These techniques can be used to delete genes (null mutation)
35 encoding for surface antigens, e.g., HLA histocompatibility and non-ABO blood group antigens, on red blood cells, platelets, and other blood cells. For example, deletion of the beta-2-microglobulin gene will

prevent expression of A, B and C antigens. In a similar manner, genes for the red blood cells antigens--Kell, Kidd, and Duffy--may, if desired, also be deleted, thereby preventing the expression of their respective antigens.

5 These genetically altered cells may then be transfused into a patient without inducing antibodies which may limit further transfusions. These cells are commonly selected (after transfection) by including a second gene construct (e.g., neomycin resistance gene) that can be utilized in

10 a positive-negative selection process (see, e.g., Mansour et al., *Nature (Lond.)* 336: 348 (1988)). The efficiency of this process may be as high as 85% (Te Riele et al., *Nature (Lond.)* 348: 649 (1990)). In addition, dominant gain of function insertion mutations may be used to treat

15 genetic diseases that manifest symptoms due solely to enzyme or protein deficiency (see, e.g., Rossant, above; Demarquoy et al., *Experientia*, 49: 345 (1993)).

Thus, the invention can also provide cells for the treatment of a wide variety of congenital or acquired

20 genetic diseases. This is accomplished by expanding cultures of genetically altered bone marrow precursor cells in bioreactors until cell numbers are large enough for reinfusion into the patient.

What Is Claimed Is:

1. A method for the in vitro production of mature human blood cells, comprising the steps of:
 - a) removing from a human donor pluripotent hematopoietic stem cells;
 - b) incubating said stem cells in a bioreactor in a growth medium;
 - c) contacting said stem cells in said growth medium with a recombinant human growth or maturation promoting polypeptide ("GMPP"), wherein said polypeptide promotes the growth of said stem cells or the maturation of said stem cells into differentiated human blood cells; and,
 - d) harvesting said differentiated blood cells.
2. A method of claim 1, wherein said bone marrow stem cells are derived from a blood type O, (Rh factor) negative human donor.
3. A method of claim 1, wherein said bioreactor comprises an artificial capillary cell culture system, a porous ceramic matrix culture system, or a protein coated microsphere culture system.
4. A method of claim 1 wherein said differentiated blood cells comprise erythroid cells, granulocytic cells, monocytic or thrombocytic cells.
5. A method of claim 4 wherein said erythroid cells comprise erythrocytes or reticulocytes.
6. A method of claim 4, wherein said granulocytic cells comprise neutrophils, eosinophils, or basophils.
7. A method of claim 4 wherein said monocytic cells comprise monocytes or macrophages.
8. A method of claim 4, wherein said thrombocytic cells comprise megakaryocytes or platelets.
9. A method of claim 1, wherein said polypeptide GMPP is selected from the group consisting of SCGF, IL-1, IL-3, IL-4, IL-5, IL-11, GM-CSF, M-CSF, G-CSF and EPO.
10. A method of claim 1, wherein said GMPP comprises a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF and EPO, and said blood cells produced are erythrocytes.

11. A method of claim 1 wherein said GMPP comprises a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF, and IL-11, and said blood cells produced are platelets.

12. A method of claim 1, wherein said GMPP comprises
5 a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF and M-CSF, and said blood cells produced are monocytes or macrophages.

13. A method of claim 1, wherein said GMPP comprises
10 a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF and G-CSF, and said blood cells produced are neutrophils.

14. A method of claim 1, wherein said GMPP comprises a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF and IL-5, and said blood cells produced are eosinophils.

15. A method of claim 1, wherein said GMPP comprises a combination of SCGF, IL-1, IL-3, IL-6, GM-CSF, and IL-4, and said blood cells produced are basophils.

16. A method of claim 1, wherein said stem cells are genetically modified so as to be substantially free of cell surface histocompatibility antigens.

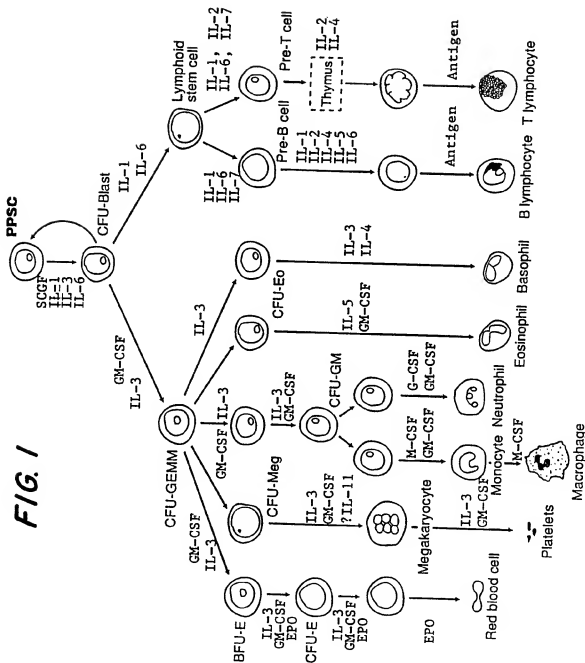
20 17. A method of claim 1, wherein said stem cells are genetically modified so as to be substantially free of cell surface blood group antigens that are incompatible with the recipient.

25 18. A method of claim 1, wherein said stem cells are genetically altered so as to produce a specific protein.

19. An improved composition for supplying iron to a subject requiring same, comprising iron-saturated hemoglobin isolated from erythrocytes produced from human universal donor pluripotent hematopoietic stem cells
30 incubated in a bioreactor in a growth medium containing a GMPP combination comprising SCGF, IL-1, IL-3, IL-6, GM-CSF and EPO.

20. A composition of claim 19, wherein said human donor is of blood group O and is Rh factor negative.

I/I



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/11810

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/00, 5/02, 5/06; A61K 35/14, 35/18

US CL : 435/240.2, 240.21, 240.25, 240.3, 240.31; 424/529, 533

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.2, 240.21, 240.25, 240.3, 240.31; 424/529, 533

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: stem cells, cytokines, expand, differentiate, interleukin, growth factors

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,004,681 (BOYSE ET AL.) 02 April 1991, see entire document.	1-20
Y	US, A, 5,199,942 (GILLIS) 06 April 1993, see entire document.	1-20
Y	US, A, 5,192,553 (BOYSE ET AL.) 09 March 1993, see entire document.	1-20
Y	Leukemia, Vol. 6, No. 10, issued October 1992, Terstappen et al., "Differentiation and Maturation of Growth Factor Expanded Human Hematopoietic Progenitors Assessed by Multidimensional Flow Cytometry", pages 1001-1010, see entire document.	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search 01 MARCH 1995	Date of mailing of the international search report 13 MAR 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer SUSAN M. DADIO <i>Nathaniel Fagan</i>
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/11810

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Experimental Hematology, Vol. 20, No. 3, issued March 1992, Muench et al., "Interactions Among Colony-stimulating Factors, IL-1 β , IL-6, and <i>Kit</i> -ligand in the Regulation of Primitive Murine Hematopoietic Cells", pages 339-349, see entire document.	1-20
Y	Bio/Technology, Vol. 11, issued March 1993, Koller et al., "Expansion of Primitive Human Hematopoietic Progenitors in a Perfusion Bioreactor System with IL-3, IL-6, and Stem Cell Factor", pages 358-363, see entire document.	1-20
Y	J. Cell Biochem., Suppl. 0, (16 Part F), issued 1992, Koller et al., "EXPANSION OF HUMAN HEMATOPOIETIC PROGENITORS WITH SYNERGISTIC CYTOKINE COMBINATIONS IN A PERFUSION BIOREACTOR", page 135, see abstract CE 302.	1-20
Y	US, A, 5,154,921 (SAGER ET AL.) 13 October 1992, see entire document, especially Figure 1.	1-20
Y	Blood, Vol. 81, No. 3, issued 01 February 1993, Srour et al., "Long-Term Generation and Expansion of Human Primitive Hematopoietic Progenitor Cells In Vitro", pages 661-669, see entire document.	1-20
Y	Biotechnology Progress, Vol. 9, issued 1993, Sardonini et al., "Expansion and Differentiation of Human Hematopoietic Cells from Static Cultures through Small-Scale Bioreactors", pages 131-137, see entire document.	1-20
Y	Golde, "Hematopoiesis", published 1984 by Churchill Livingstone (New York), pages 71-85 (chapter 2), see entire chapter.	1-20